The University of Chicago

THE RESPONSE OF GUINEA PIG MAMMARY GLANDS TO INJECTED SEX HORMONES AND OVARION GRAFTS AND ITS BEARING ON THE PROBLEM OF SEX HORMONE ANTAGONISM

A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY
1932

By
GEORGE KEISER SMELSER

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THE RESPONSE OF GUINEA PIG MAMMARY GLANDS TO INJECTED SEX HORMONES AND OVARIAN GRAFTS AND ITS BEARING ON THE PROBLEM OF SEX HORMONE ANTAGONISM[1]

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(Twelve figures)

GEORGE K. SMELSER
Hull Zoölogical Laboratory, University of Chicago

INTRODUCTION

THE biological potentialities of the mammary glands present many interesting problems, such as their origin, their differentiation, their growth, and regulation of their normal function of lactation by hormones. The present investigation involving primarily a study of their response in growth, differentiation, and function to various hormonal conditions was undertaken with the hope of obtaining additional information relative to their response to various hormone influences.

Since Steinach (1912) introduced his concepts of the influence of sex hormones in the organism, several points of special interest have arisen. A brief review will serve to illustrate the lack of agreement among investigators as to the extent and type of sex hormone influences.

Steinach (1912) first demonstrated, chiefly by gonad grafts, that the majority of the secondary sex characters were controlled by secretions from the homologous sex organs. Thus male structures, such as the penis, seminal vesicles, prostate glands, etc., were dependent upon the testis for their state of growth and function, while female structures, including mammary glands, were largely controlled by the ovary. These conclusions have received almost universal substantiation in work since that time.

A second contention of Steinach, however, has been severely questioned. He maintained that sex hormones, in addition to stimulating homologous sex characters, inhibited the sex characters

[1] This investigation has been aided by a grant from the Committee on Research in Problems of Sex of the National Research Council; grant administered by Professor Frank R. Lillie.

of the opposite sex. This hypothesis was based in part upon the fact that gonads transplanted into normal animals of the opposite sex did not persist, nor did they show any hormal effect. On this basis he postulated a "sex antagonism" that extended to the gonad tissue itself. When an animal was previously "desexed" by removal of its gonads, subsequent or simultaneous transplantation of gonads of both sexes led to persistence and some endocrine function of each.

Sand (1918) was the first to point out the necessity of modifying Steinach's concept of antagonism. He was able to obtain persistent and functioning ovarian grafts when the ovary was transplanted into the testicle of the male guinea pig. He postulated the presence of some food substance in the blood stream necessary for normal gonad function which was removed from the blood stream and stored in the gonad. Gonads grafted intramuscularly or to the peritoneum of the opposite sex were unable to obtain this substance in sufficient amounts to become active, whereas ovaries grafted into the testis could obtain the necessary substance. Such grafts were successful in both rats and guinea pigs. In the latter the ovarian tissue was quite normal and its endocrine influence proved by growth and function of the mammary glands. In rats this ovarian endocrine activity was not demonstrated, for a rudimentary nipple is lacking in the male. These experiments are the basis for Sand's hypothesis. It should be emphasized that, in cases where endocrine activity of the ovary was revealed, one or both testes showed degenerative changes in the tubules. This was occasioned by adhesions between the testes and the abdominal or inguinal peritoneum following the operation. Sand apparently does not attach great import to this atrophy. These experiments and also those of Moore (1921*a*) and Lipschütz (1925*a*) clearly demonstrate that successful heterosexual gonad grafts can be made without previous or simultaneous castration of the host.

Lipschütz, from 1918 to 1929, has worked extensively with phenomena that have added further complications to the hormone antagonism question. He was able to transplant ovaries successfully into the kidneys of normal male guinea pigs, but such ovaries usually failed to exhibit evidence of hormone secretion or did so only after a very long time. Thus ovaries placed in the kidney of a cas-

trated male or female animal gave evidence of endocrine activity within a period of approximately two weeks, for at this time the mammary glands of the host began a rapid growth that continued perhaps for some months. In the kidney of the normal male, however, ovarian grafts though well incorporated did not stimulate growth of the mammary glands until a long period of latency had intervened. Experiments conducted by Lipschütz in Esthonia placed this period of latency of mammary hypertrophy in normal males bearing ovarian grafts at from five to eight months (1925*a*), but later ones done after his arrival in Chile reduced this period of latency to approximately four to seven weeks (1927*b*). Such delayed endocrine function on the part of ovarian transplants was considered to be due to an antagonistic influence from the testes.

Further evidence for hormone antagonism was found by Lipschütz (1925*b*) in the sudden appearance of mammary gland growth following castration. Thus a normal male might be host to an intra-renal ovarian graft for a period of five months without external evidence of its presence, but, if the testes were removed, mammary growth would become evident within two weeks and growth would continue for several months. Lipschütz termed such a response the "unbolting reaction," visualizing that removal of the testis suddenly released the ovarian graft from its suppression and permitted its function. However, removal of the testis was not essential as either reduction in mass or elevation of testes into the abdomen (experimental cryptorchidism) served equally well to release the ovarian graft from the antagonistic influence of the normal testicle.

It should be pointed out that the differences of opinion just reviewed came from observations derived from studies in which the surgical method of treatment was almost exclusively used. The utilization of purified hormone preparations, it would seem, should lead to more nearly similar ideas, but it has not proved so. Thus Steinach has recently renewed his earlier contentions of sex hormone antagonism to interpret the results of injecting ovarian extracts into male animals (Steinach and Kun, 1926 and 1931). Normal male rats injected with the female sex hormone (oestrin, menformon, etc.) were found to exhibit not only damage to the testes but a degenerate condition in all the reproductive accessory organs.

Moore (1919, 1920, 1921*a*, *b*, 1930*a*) has denied repeatedly that conclusive evidence has been presented for antagonism between the two sex glands. In a long series of experiments he has been able to show for both sexes of rats and guinea pigs that heterosexual gonad transplants were easily obtained when the host's own gonads were present. Testis grafts were recovered months after transplantation into normal females and were carrying on spermatogenetic activity despite the fact that such females had mated, delivered, and suckled young. Similarly, ovarian grafts showing normal follicular development were recovered from males that had inseminated females during the period of graft persistence.

Moore and Price (1932), while in entire agreement with others that the female hormone (oestrin) causes damage to the male reproductive tract when administered through subcutaneous injection, pointed out that administration of testis hormone to normal young males induced testicular damage of a similar grade and character to that which followed oestrin treatment; they hold that the condition developed after each treatment must have a common basis of explanation and such a basis cannot rest upon an antagonism between the two sex hormones. It was shown by them that a given dosage of testis hormone just sufficient to maintain the male accessory reproductive organs in a normal condition would have entirely similar effects if injected alone, or accompanied by a dose of oestrin previously found highly injurious to the testes of males. Rather than an antagonism between the two hormones Moore and Price explain their results on the basis of a reciprocal gonad-hypophysial interaction. Oestrin is injurious to the male reproductive tract not because it is antagonistic to the male system or secretions from it but rather because oestrin suppresses the activity of the hypophysis in such a manner that the organism is no longer provided with the necessary hypophysial secretions for gonad maintenance and function, hence damage to the male system by oestrin is similar to that which follows hypophysectomy. Their contentions are supported by numerous lines of evidence.

In view of the radically different existing opinions, further investigations in the field of hormonal influences within the organism are to be desired. In the following pages will be given the results of an

investigation carried on for the past three years in which the reactions of the mammary glands of the guinea pig to various hormonal states within the organism have been followed. The ordinary surgical treatments and the injection of hormone preparations have been combined in an attempt to define the limits of response of these structures to the different conditions imposed upon them.[2]

MAMMARY GLAND RESPONSES IN NORMAL AND GONADECTOMIZED GUINEA PIGS

The mammary glands though most often considered as secondary sexual characters of the female are differentiated equally in both sexes of the guinea pig. At birth in either the male or female the nipples are well defined and approximately 1 millimeter in height. The gland tissue proper consists of branched tubules located in the dermis of the skin and has a circular spread of approximately 1–2 centimeters in diameter. Histologically the tissue of these structures is as abundant and as well developed in the male as in the female.

In normal females repeated measurements of the nipples show clearly that a slow growth occurs from birth to puberty. At the time of the first oestrum, or perhaps a few days earlier, the nipples become quite turgid and from this time a more rapid growth ensues. At six months of age their height is of the order of 4 or 5 millimeters and little further increase is evident excepting during pregnancy and

[2] This problem was suggested to me by Dr. Carl R. Moore, and it is with great pleasure I acknowledge my indebtedness to him not only for a most interesting problem but also for his constant guidance and encouragement.

The material requirements for an investigation of this nature are numerous, and the investigation itself could not have been conducted without the hearty co-operation of others. To Professor R. G. Gustavson, of the Department of Physiological Chemistry of the University of Chicago and the University of Denver, I am indebted for preparations of the female hormone, oestrin, prepared from human placentae and human pregnancy urine. The preparations had been carefully assayed by the method outlined by Coward and Burn (1927). To Professor F. C. Koch and Dr. T. F. Gallagher, Department of Physiological Chemistry of this University, I am indebted for preparations of testis hormone prepared from extracts of bull testes and carefully assayed by Dr. Gallagher by the capon comb method (see Gallagher and Koch, 1930). I also wish to thank Dr. O. Kamm of Parke Davis Company for preparations of anterior pituitary extracts. A great number of the 500 guinea pigs employed in this study were kindly placed at my disposal by Dr. Sewall Wright from his large colony maintained in connection with experiments in genetics. It is indeed a pleasure to express my gratitude for these invaluable aids.

lactation (see Fig. 1). The hormones of pregnancy cause marked changes both in the nipple height and in glandular differentiation. Though a great deal of variation is found in nipple height of pregnant females the usual state is one of approximately 8–10 millimeters. During the active nursing period this height is increased, probably due to the mechanical factors in suckling rather than to hormonal ones. After weaning a decided regression occurs, but usually the nipples of females that have given birth to young remain at a level above those of virgin animals.

The mammary glands of young males experience a similar slow growth to those of females up to the stage of puberty, but there is no

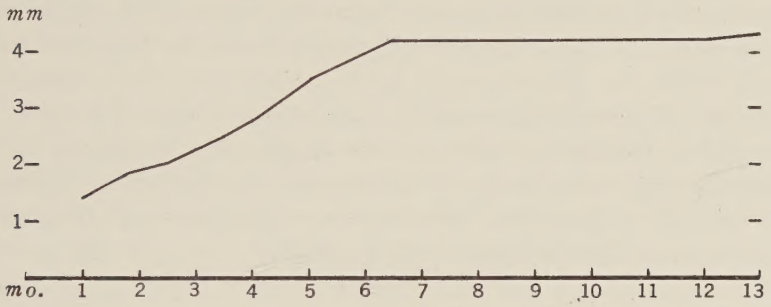

FIG. 1.—Growth of nipples of normal females. Each point is an average of 35 individuals.

marked increase either in nipple height or growth of the secreting portion beginning at that time. Neither is there any evidence of regression as the testes become functional.

Removal of the ovaries introduces marked differences in the condition of the mammary glands of the females. When ovariotomy is performed before the stage of puberty is reached there is no evidence of a growth acceleration either in nipples or in the tubules or acini of the glandular portion. In fact, the conditions are entirely comparable to those found in the normal male. When the ovaries are removed at the age of six months, when sexual maturity has been reached, the regression occasioned in the glandular tissue is much more rapid than that of the nipples; the latter may fail to exhibit regression for a period of four to five months.

Removal of the testes occasions no changes in the mammary ap-

paratus of males. The glands or nipples of prepubertally castrated males differ in no appreciable manner from those of normal males. Castration in adults likewise fails to introduce any modification in these structures.

It therefore becomes apparent that the mammary gland of the guinea pig is a somatic structure, present at birth in both sexes, that fails to exhibit any evidence of sexual dimorphism. Its growth and further development is stimulated by hormones derived from the ovary, but it appears to be insensible to hormones from the testis. Since prepubertal castration fails to introduce changes in its structure, it is evident that the rudimentary condition found in the males is due only to lack of substances that will stimulate its development, rather than to the presence of substances that arise from the testis and inhibit its development. Early ovariotomy and castration renders the mammary apparatus of both sexes identical so far as I am able to determine. Structurally it appears that the glands of the two sexes are equipotential, but recourse must be had to injection of hormone preparations before one is justified in concluding that they possess a physiological equipotentiality.

MAMMARY GLAND RESPONSE TO HORMONE INJECTIONS

EQUIPOTENTIALITIES

My chief interest in a study of the control of the mammary glands concerns not only the question of their equipotentiality in the two sexes but also whether their potentialities are in any manner affected by hormones from the testis. It was early appreciated that information regarding either problem would require some approach to quantitative methods of study.

Three methods of study of the mammary glands have been investigated and all applied to some extent; in some cases all three have been utilized for the same material. A histological study of the glandular tissue has some advantages and many disadvantages. Whereas the study of sectioned material shows more clearly the actual state of the secreting tissue, and one can readily distinguish the normal female gland from that of the normal male, still it is with great difficulty that one can apply quantitative methods to dis-

tinguish different grades of activity. The study of the dissected gland as a whole mount preparation has certain advantages over that of sectioned material. Thus when the gland is fixed in 10 per cent formalin and stained *in toto* with dilute haematoxylin the glandular tree is very apparent among the fat and connective tissue. However, despite the fact that the spread of the glandular portion gives a more adequate comparative picture, it is difficult to obtain any satisfactory measure of the response because of the lack of compactness of the tissue. The most satisfactory index of activity proved to be the mere measurement of the nipple height. It was found that nipple height paralleled to a large degree the glandular differentiation, and since it could be repeatedly taken without sacrifice of the animal, or a single gland, it has served as the chief index of response.

In order to compare the reaction of the mammary glands of males and females, it required first to ascertain the threshold of response to oestrin. In preliminary experiments, therefore, different dosages of oestrin were injected subcutaneously each day for twenty days into adult castrated male animals. Since the glands had remained entirely quiescent from birth, and since the testis hormones were eliminated through castration, the response of the glands would start from a base level. Measurements of nipple height were taken throughout the experiment and the gland tissue was preserved and studied in histological preparations. The results obtained from this preliminary investigation revealed the fact that a definite response in growth of the mammary apparatus occurred with a daily injection of much less than one rat unit of oestrin, the rat unit corresponding to the Coward and Burn designation (1927).

Fifteen adult male guinea pigs, castrated from four to six weeks preceding a more detailed experiment, were divided into lots of 3 each. These lots were given daily injections of (*a*) olive oil, (*b*) 0.05 rat unit of oestrin, (*c*) 0.125 rat unit of oestrin, (*d*) 0.25 rat unit of oestrin, and (*e*) 0.375 rat unit of oestrin. The samples were so arranged that each animal received the same quantity of olive oil as the control group. Injections were continued for twenty days during which time nipple height was repeatedly determined and the mammary gland tissue was preserved and sectioned at the termination

of the experiment. The results obtained from nipple height measurement are shown in Figure 2.

The sectioned glands were of little value in determining relative responses to these small dosages of oestrin but were of general confirmative value for the nipple response. Examination of the figure, however, shows the definite responses given by nipple growth. Daily injection of 0.05 rat unit of oestrin is the minimal amount to which a response is obtained, whereas dosages of 0.125 and 0.25 rat unit produce perfectly definite growth responses. With a knowl-

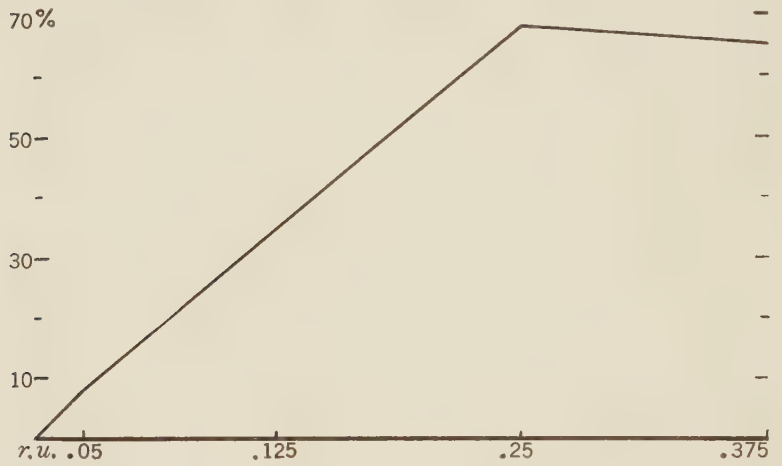

FIG. 2.—Growth response of nipples of adult castrate males to 20 daily injections of oestrin. Each point represents the average percentage of increase in nipple height of three animals to a given concentration of oestrin injected. The dosages are .05 .125, .25, and .375 rat unit.

edge of such responses on the part of the glands from castrated males, a comparative study was made of the relative sensitivity of these structures in the two sexes.

A group of 18 males and 18 females were subjected to gonadectomy before thirty days of age and permitted to grow to adults. They were then divided into three groups consisting each of 6 males and 6 females. Group (*a*) was given daily injections of 0.125 rat unit of oestrin, group (*b*) 0.25 rat unit, and group (*c*) 0.5 rat unit of oestrin. The experiment was carried through a period of thirty-five days, and nipple measurements were taken regularly (see Fig. 3).

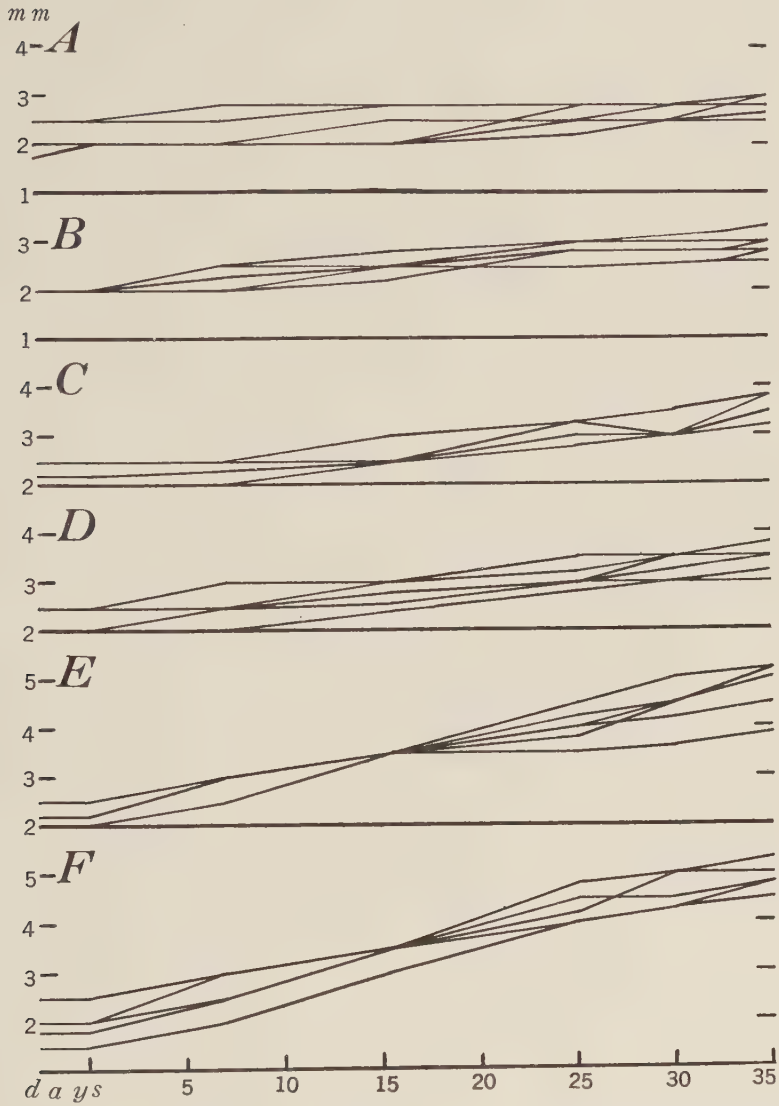

FIG. 3.—Growth response of nipples of adult castrated males and spayed females (prepubertal gonadectomies) to various concentrations of oestrin injected for 35 days. *A*, six spayed females receiving .125 rat unit daily; *B*, six castrated males receiving .125 rat unit daily; *C*, six spayed females receiving .25 rat unit daily; *D*, six castrated males receiving .25 rat unit daily; *E*, six spayed females receiving .5 rat unit daily; *F*, six castrated males receiving .5 rat unit daily.

An examination of the figure reveals the fact that nipple height as recorded runs parallel to the amount of oestrin injected. It is a simple matter to determine the groups receiving the different dosages; although some variability among the groups was noted it is always of such an order as to be of no other significance than minute individual variability. Of particular importance is the fact that the response of the male nipples was in no manner different from that of the females; the nipple response of males is just as sensitive to the injected oestrin as is that of females.

The data presented in this and the preceding section permit the conclusion that the mammary gland of the guinea pig is a somatic structure that is equipotential, anatomically and physiologically in the two sexes. It is affected by, and responds to, oestrin but is apparently unaffected by hormones from the testis. The young animals do not exhibit a sex dimorphism in the mammary glands, and the dimorphism expressed in adults is merely the result of the response of the female glands to oestrin; the lack of response on the part of the male gland is due to the lack of ovarian secretions rather than to the presence of testicular secretions.

MAMMARY GLAND RESPONSE TO SEX HORMONE MIXTURES

It was demonstrated in the preceding section that the mammary glands of the males and females were equally sensitive to the injection of oestrin. For the purpose of determining whether the presence of testicular hormones modified in any manner the response to oestrin, the mammary changes were studied in both males and females that received injections of oestrin alone and of oestrin accompanied by effective doses of testis hormone. The experiment involved the utilization of 32 animals all of which were twelve months old; 11 males and 12 females of this group had been gonadectomized before puberty. The groups and treatment were as follows: (1) 3 normal males—oestrin and testis hormone, (2) 3 normal males—oestrin alone, (3) 4 castrated males—oestrin and testis hormone, (4) 4 castrated males—oestrin alone, (5) 4 spayed females—oestrin and testis hormone, (6) 4 spayed females—oestrin alone, (7) 3 castrated males—olive oil, (8) 3 normal males—olive oil, and (9) 4 spayed females—olive oil. Each animal was given daily injections for a

period of twenty days and the injected material was so compounded that each animal received the same quantity of olive oil as the control group injected with olive oil alone. The quantity of oestrin was in every case 0.2 rat unit daily but those animals receiving the mixed hormone injections had in addition seven bird units of testis hormone. This amount of testis hormone had been shown previously to be a dose effectively substituting for the testis (Moore and Gallagher, 1930; Moore and McGee, 1928). Moreover, its potency was proved in these experiments by the fact that the prepubertally castrated males, when a year old, responded on the tenth day of the mixed hormone injections by giving a coagulable ejaculate after electrical stimulation on the head (for the method see Moore and Gallagher, 1930). Nipple measurements were made before injections were begun and on each five succeeding days. At the end of the twenty days of injection the mammary glands removed for sectioning afforded material that confirmed the response indicated externally in nipple growth. The observations on nipple measurements are expressed in Figure 4 as averages for the different groups involved.

A study of the figure reveals several points of prime importance which were confirmed by a study of the sections of these glands.

In every case, considering the groups as a whole, the mammary glands of animals (male or female) receiving effective doses of testis hormone along with the oestrin showed as great nipple growth as did those receiving the same dosage of oestrin alone. In fact, two of the groups showed slightly more growth after the administration of both hormones. Since the mixture of the two hormones was made at the beginning of the experiment, sufficient time was given for the occurrence of any chemical interaction that might be conceived possible; hence the absence of any modification of the potencies of oestrin by the testis hormone indicates clearly the absence of chemical antagonism between these two substances. Furthermore, the fact that responses of the mammary apparatus were as great in the presence of effective doses of testis hormone as when oestrin alone was administered proves beyond doubt that the testis hormone is in no manner a deterrent to the responses of the glands to the homologous hormone stimulation.

The conclusion is justified, therefore, that sex hormone antagonism does not exist if one may understand antagonism to mean either a chemical interference or neutralization between the two substances or the interference of one upon the normal reaction of the end organ to its homologous hormone. Rather does the end organ (in this case the mammary glands) respond to its normal stimulating substance, or homologous secretion, and exhibit no response to the heterologous secretions either as a stimulating effect or an inhibiting effect.

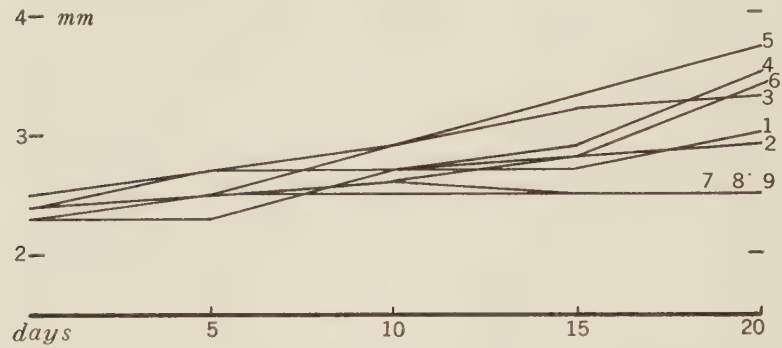

FIG. 4.—Growth response of the nipples of adult normal and castrated males and spayed females to oestrin and mixtures of oestrin and testis hormone. Animals receiving oestrin only were injected with .2 rat unit daily. Those receiving the mixture were injected with .2 rat unit of oestrin plus 7 bird units of male hormone in olive oil daily. A third group received only olive oil. 1, Three normal males receiving oestrin and testis hormone; 2, Three normal males receiving oestrin; 3, Four castrated males receiving oestrin and testis hormone; 4, Four castrated males receiving oestrin; 5, Four spayed females receiving oestrin plus testis hormone; 6, Four spayed females receiving oestrin; 7, Four castrated males receiving olive oil; 8, Three normal males receiving olive oil; 9, Four spayed females receiving olive oil.

MAMMARY GLAND RESPONSES TO OVARIAN GRAFTS

The preceding sections have presented observations that are fundamental in an interpretation of the responses of the mammary glands in both sexes to the stimulating action of oestrin. The chief difficulty in understanding completely the contentions of those who maintain the view of an antagonistic action between the hormones involves the interpretations of observations made as a result of ovarian transplantation. It appeared desirable, therefore, to reinvestigate the problem of the effects of ovarian grafts in different types of animals.

The method employed in making ovarian grafts for this investigation is a modification of the one first described by Marshall and Jolly (1907) and later employed by Lipschütz (1925*a*); this involves for the most part the transplantation of an ovary into the kidney where a particularly good vascular bed materially aids the establishment of vascularity. After clipping, shaving, and sterilization, an incision is made through the skin lateral to the mid-dorsal line. Epaxial and hypaxial muscles are separated and the dorsal portion of the kidney exposed. The kidney is slightly elevated by inserting a needle into its substance; and the ovarian tissue, threaded bead-like on short lengths of No. 00 catgut, is drawn against the kidney tissue, the needle passing tangentially through a small portion of the organ. An incision is made with a narrow scalpel blade quite deeply into the kidney alongside the needle, and the ovary, previously scarified with a needle point to aid blood-vessel ingrowth, is pulled into the substance of the kidney where it is held firmly by traction of the catgut. Removal of the needles, return of the kidney to its normal position, and closure of the sutures completes the operation which with practice can be performed in approximately ten minutes. The donor ovaries were obtained from sacrifice of normal healthy females, or at simultaneous operation, and were never separated from the donor for more than a few minutes previous to incorporation in the host. The amount of ovarian tissue transplanted has varied from one-half to two entire ovaries, but no significant difference has followed the variable amount implanted; hence they will be treated as if a single whole ovary had been utilized.

Ovaries have been transplanted into more than 270 animals, and these animals have been carefully examined for periods usually of from five to six months; some, however, were under observation for more than a year. The reactions of the mammary glands have been followed closely and the nipple height recorded frequently throughout the course of the experiment.

OVARIAN GRAFTS IN ADULT NORMAL MALES

Intrarenal ovarian grafts.—Ovaries have been transplanted into 150 adult normal males. Ninety-five per cent of these host males failed to show any mammary gland hypertrophy over a period of four to six months. Subsequent to this period 100 of the males were

subjected to some testicular manipulation. Among these, 75 involved the transfer of the testes from the scrotum into the abdomen (experimental cryptorchidism) and 25 involved castration. Twenty of the 100 responded immediately by increased growth of the nipples. A few examples of these responses to illustrate the various reactions observed appear advisable.

Table I will serve to illustrate the type of response occurring after delayed castration.

TABLE I

PROTOCOL OF A NORMAL MALE (NO. 475) HOST TO AN OVARIAN GRAFT

Nipple Height in Millimeters		Date	Remarks
Left	Right		
2.5	2+	3–19–31	Received intrarenal ovarian graft. Donor 7 months old. Host 8 months old
2.5	2+	4–10–31	
2.5	2+	5–30–31	
2.5	2+	7–17–31	
2.5	2+	8–13–31	Castrated. Testes normal, motile sperm in epididymis
2.5	2+	8–20–31	
8.0	8.0	8–31–31	
13.0	12.0	10–31–31	
13.0	12.0	2– 4–32	Died. Ovarian graft preserved.

Variations in nipple response are to be expected, and were noted; the observations in Table I are characteristic for this general type of response. A slow growth of the nipples in normal males occurs from birth to maturity but this growth is very slight in comparison with that occurring in the normal female. Since a similar growth occurs in castrated males or females, it is to be attributed to general body growth rather than to a response to hormonal influences. In the above-cited case the normal male eight months old showed a nipple height of approximately 2 millimeters. For a period of five months after ovarian transplantation the nipples failed to give evidence of the presence of the graft. Within two weeks after removal of the testis, however, the nipples had increased from a height of 2 millimeters to 8 millimeters, and continued to grow for a period of two months, finally reaching a height of 13 millimeters.

In such cases as this, therefore, the absence of mammary reactions cannot be attributed to the failure of incorporation of the graft.

That it was present and capable of function is attested by the fact that immediately upon testis removal mammary growth began and progressed rapidly. It should be further noted that virgin females of similar age exhibit nipple heights of approximately 4–5 millimeters whereas the male cited above produced nipples 13 millimeters in height. It becomes clear, therefore, that the presence of the testis in some manner interferes with the endocrine secretory function of a well-incorporated ovarian graft. This type of reaction has been termed the "unbolting reaction" by Lipschütz (1925*a* and *b*) and was considered by him to give evidence of an antagonistic action of the testis upon the ovarian graft. But in the previous sections evidence has been presented that an antagonism between the internal secretions, as we have used these terms previously, is in no way indicated. Some other explanation must be given to account for the conditions observed.

Among the group of normal males receiving ovarian grafts, 6 males were observed to experience nipple hypertrophy with but little interference by the testes. Nipples began a slow growth in these 6 animals at varying times after ovarian transplantation, but growth was neither so rapid nor so pronounced as that occurring after castration. Evidently whatever influence the testes exert in preventing the activity of the graft was only partially operative; nipple height in such cases was never greater than 7 millimeters. At autopsy the testes of such males were entirely normal in appearance and histology, and the male accessory organs were fully developed. The testes were active both in gametogenesis and in hormone production. The protocol given in Table II illustrates one such case.

A second type of testicular manipulation was the removal of the testes from the scrotum and confinement within the abdomen. Such an operation in male guinea pigs is invariably followed by marked degeneration of the testes and the ultimate loss of all the gametogenetic portion of the testis, but no modification of the endocrine activity of the organ occurs (see Moore, 1924). Lipschütz (1925*a*) has reported such a testis manipulation to be equally as effective as castration in the removal of the inhibiting influences of the testicle on an ovarian graft.

Among the group of normal males bearing intrarenal grafts, 36

animals, which for months had failed to exhibit mammary responses to the ovarian grafts, were subjected to the above type of operation. Of this number 12 showed an immediate response of nipple growth and those which failed to respond were later castrated, also without a response; search for the grafts at a slightly later period showed them to be degenerate and represented only by connective tissue.

These cases confirm the contentions of Lipschütz and others that loss of the germinal portion of the testis through abdominal elevation removed the influence from the testis that interfered with

TABLE II

PROTOCOL OF A NORMAL MALE (NO. 357) HOST TO AN OVARIAN GRAFT

Nipple Height in Millimeters		Date	Remarks
Left	Right		
1.5	1.5	10– 6–30	Received intrarenal ovarian graft. Host 6 months old. Donor 50–60 days old
4.0	4.5	11–18–30	
6.5	6.5	12–10–30	
7.0	7.0	12–23–30	
6.5	6.5	1– 7–31	Sired normal litter
6.0	6.0	1–23–31	

ovarian endocrine function. However, the loss of the germinal epithelium in no manner interferes with the hormone secretion of the testis, at least with that portion of the hormone that controls the male accessory reproductive organs; it is obvious, therefore, that further interpretations are necessary (see discussion).

Intra-testicular ovarian grafts.—Sand (1918) transplanted ovaries into the testes of guinea pigs and noted their endocrine influence upon the mammary glands, but a careful survey of these protocols indicates that, in every case in which the ovarian graft was functional, testicular degeneration was also involved.

During the course of my study, operations were made in which ovaries were placed within the testicular substance in 23 normal adult males. The operation must be carefully done if one wishes to avoid the complications of testis degeneration due to adhesions and approximation of the testis to the higher temperatures of the abdomen, which are injurious to the germinal epithelium. In all cases

the testes were drawn from the scrotum through a low median ventral incision in the body wall. A small incision through the *tunica albuginea* permitted insertion of an ovary into each testis. The subsequent careful repair and the return of the testis to the uninjured scrotum resulted in a few cases in which no adhesions or scrotal disturbances were involved.

In all cases where the testes remained within the scrotum without adhesions and without injury to the germinal epithelium, the mammary gland response was negative. Subsequent elevation of the testes containing the ovary grafts from the scrotum into the abdomen, however, brought forth the mammary response in every case in which a graft could be recovered at autopsy. Furthermore, all graft-bearing animals in which the operation led to the formation of adhesions and moderate injury of the gametogenetic tissues of the testis showed pronounced mammary growth.

From these data it becomes clear that ovarian grafts in the kidney, or testis, of normal adult males become successfully incorporated and persist for months without being able to secrete hormone. That this hormone is not being produced or released, rather than that it is destroyed or rendered ineffective, is shown by the fact that injections of minute quantities of the hormone subcutaneously into normal males bring on immediate responses of the mammary apparatus. Conditions that lead to injury or loss of the germinal epithelium of the testis such as castration, or experimental cryptorchidism, make it possible for a graft, which has long remained inactive, to become functional in an endocrine sense almost at once.

Despite the enormous increase in the mammary gland tissue and nipples, there were no cases among the adult normal males in which lactation occurred.

OVARIAN GRAFTS IN CASTRATED MALES

It was shown in the preceding section that ovarian grafts in normal male animals usually failed to exhibit any endocrine function despite the successful incorporation of the graft. In a few cases, slight mammary hypertrophy occurred after some delay. Invariably, when graft incorporation was successful, testis removal or the induction of some injury permitted a response in the mammary

tissue. In comparison with this result, that obtained after transplantation of ovaries into castrated males is markedly different.

Forty-five young males were castrated at thirty days of age and when they had attained an age of five to six months one ovary from an adult female was grafted intrarenally into each. In no cases during the two weeks immediately succeeding transplantation was there any appreciable growth of nipples. During the second two weeks following transplantation 50 per cent of these castrated males exhibited appreciable mammary growth. All animals were carefully followed for a period of months, with frequent nipple measurements, but there were no cases in which mammary growth occurred that was not perfectly definite within the second two weeks. Upon examination at autopsy all males that had failed to exhibit mammary responses were without ovarian grafts; the site of transplantation was usually marked by scar tissue, but the graft had undergone resorption in all cases.

The record of nipple growth in a few selected cases of this group is presented in graphic form (Fig. 5). It is to be noted that nipple height reached 15 millimeters in some animals within a period of four months after transplantation. This is to be contrasted with a nipple height in adult normal virgin females of approximately 5 millimeters. It is an interesting fact that mammary growth is so much greater in these castrated males bearing ovarian grafts than in normal females, and this point is important in attempting an analysis of the activities of an ovarian graft in different animals. But despite the excessively developed nipples and the hypertrophy of glandular tissues, lactation occurred in only one of these castrated males and secretion was scanty in this case. Lactation under such conditions has been treated as the usual occurrence by several authors—Lipschütz (1926), Sand, (1922*c*), Athias (1916), Pettinari (1925), Steinach (1913, 1916), etc.—but such contentions are not in agreement with my own observations. This discrepancy will be dealt with more fully in a following section.

In conclusion, it is definitely shown that ovarian grafts are readily incorporated in a castrated male host and that mammary response is very rapid. Larger amounts of oestrin are produced by ovarian grafts in castrated males than in normal males, and even larger

amounts than in ovaries of normal female animals, as evinced by the much greater growth of nipples.

OVARIAN GRAFTS IN EXPERIMENTAL CRYPTORCHID MALES

In a preceding section it was shown that when normal male guinea pigs carried intrarenal ovarian grafts that failed to liberate

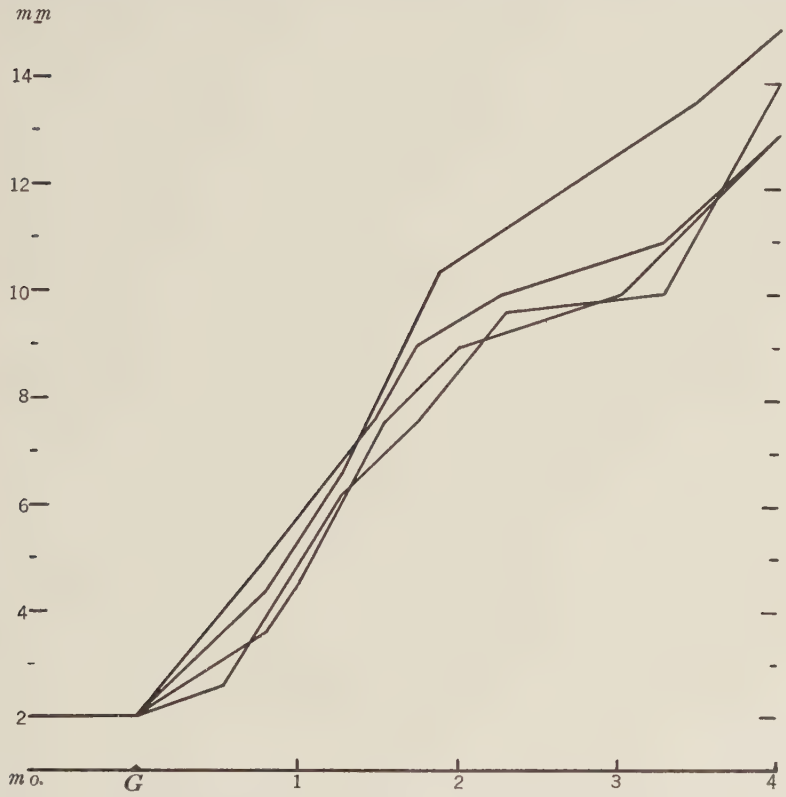

FIG. 5.—Growth response of the nipples of adult, prepubertally castrated males to ovarian grafts.

demonstrable quantities of oestrin, such a liberation occurred very shortly after elevation of the normal testes into the abdomen. In such an example of his "unbolting reaction" Lipschütz (1926) postulated the possible release of products from germinal epithelium destruction that might in some manner be responsible for removal of the inhibition exercised by the normal testicle. Since the question

deserved further consideration it appeared desirable to study the graft reactions in animals that had existed for long periods of time with testes in their final degenerate condition, rather than during the rapid and severe involution of the germinal epithelium.

Forty young males of thirty to forty days of age were subjected to experimental cryptorchidism. The testes were removed from the scrotum by severing the gubernaculum and the mesentery of the vas deferens, and were confined in the abdomen by closure of the inguinal canals. The animals received intrarenal ovarian grafts at the age of five to six months. The testes at such a period of abdominal confinement are small, usually totally devoid of germ cells, and it should be mentioned that abdominal confinement at the age given insures that the animal had never possessed a completed germinal epithelium; such animals never produce spermatozoa and the tubules of the testis contain only Sertoli cells. Interstitial tissue remains unaffected.

Approximately 60 per cent of this group of 40 males showed definite mammary hypertrophy within a period of fourteen days after ovarian transplantation. This hypertrophy was pronounced and rapid and reached its zenith within approximately two and one-half months. A few selected cases are shown in Figure 6 to illustrate the rate and intensity of nipple hypertrophy. A comparison with Figure 5 (ovarian grafts in castrated males) will demonstrate the very close similarity of the growth curves; one can quite successfully superimpose the figure of one group upon the other. And again, despite the enormous mammary hypertrophy, there were no cases in which lactation occurred.

The presence of an abdominal testis, therefore, is without effect upon the function of the ovarian grafts since essentially identical results are obtained from transplantation into castrated males and males possessing testes confined to the abdomen for a period of months. The common factor in these two groups is the absence of a germinal epithelium, however, and not the absence of testis hormone. When one examines the accessory reproductive organs of the experimental cryptorchid males bearing functional ovary grafts, it is found that they are normal in size and in storage of secretion. Moore and Gallagher (1930) demonstrated by means of the electric ejacu-

lation test that experimental cryptorchid males produced as large quantities of ejaculate as do normal males, and Jeffries (1931) showed that experimental cryptorchid rats possessed entirely normal accessory reproductive organs.

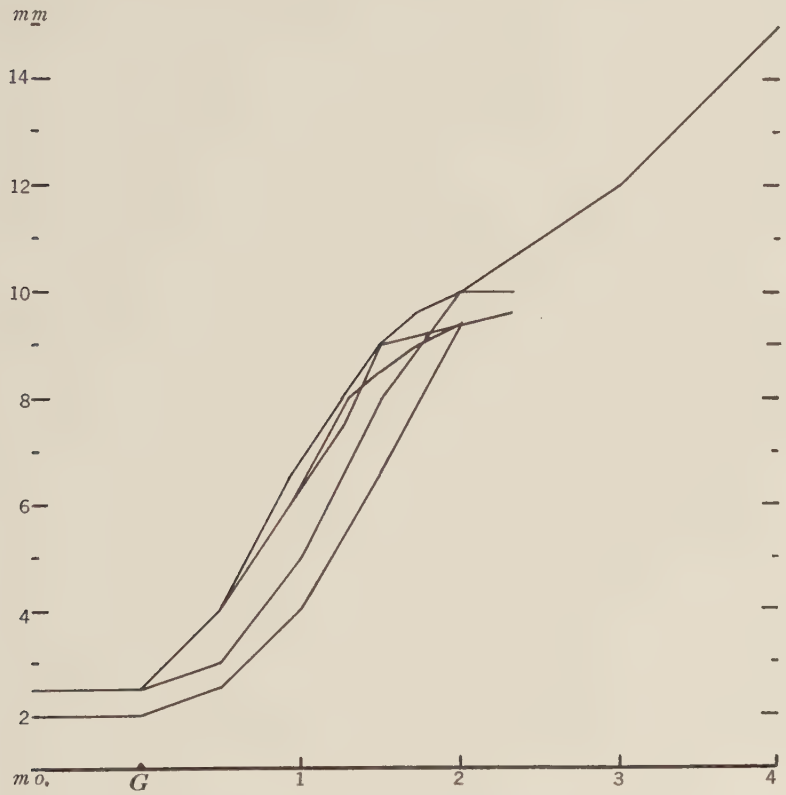

FIG. 6.—Growth response of the nipples of experimental cryptorchid males to ovarian grafts. Testis operations were made prepubertally.

It is certain, therefore, that the inhibitory influence of the testis on an ovarian graft is in no measure due to the secretions it produces that are effective in controlling the accessory reproductive organs.

OVARIAN GRAFTS IN SPAYED FEMALES

The fact that mammary glands in castrated males and experimental cryptorchid males bearing ovarian grafts were much larger

than those of normal virgin females (see previous sections) introduced the question of whether the cause lay in a different soma or in a different reactive capacity of a grafted and normal ovary. The question should be answered by the utilization of a female soma as nearly comparable to the males employed as is possible.

Thirty-five female guinea pigs were subjected to bilateral ovariotomy when thirty days of age, and when they had reached an age of five to six months ovaries were transplanted into the kidneys. They were thus as nearly comparable to the castrated males as was possible inasmuch as they were gonadectomized and received transplants at similar ages, and the nipples had remained in an undeveloped state equivalent to that of the males.

From the 35 spayed females receiving ovarian transplants, 20 showed a stimulation of the mammary apparatus within a period of twelve to fourteen days; all showing a mammary response at any time gave a definite measurable increase in nipple height within the first month after transplantation. The nipples became turgid and vascular, and grew rapidly for a short period, but soon growth was less active and they remained greatly underdeveloped in comparison with those of the treated males. The extreme nipple height noted in castrated males was 15 millimeters whereas the greatest nipple height in the spayed females was 7 millimeters. Normal virgin females of similar ages with a nipple height of approximately 5 millimeters show that, whereas nipple growth in the ovarian transplanted, spayed females was greater than normal, it was yet much below that attained by the castrated, ovarian transplanted males. The results as expressed in Figure 7 from a few selected cases should be compared with Figures 5 and 6. The causes of these peculiar differences are discussed in a later section.

Oestrous cycles.—It should be of interest to mention the oestrous behavior of these spayed females bearing functional ovarian grafts. The vaginal membrane opened for the first time just at the onset of mammary hypertrophy, twelve to fourteen days after transplantation. Vaginal smears taken twice daily according to the technique of Stockard and Papanicolaou (1917) were carefully compared with smears made from a group of normal females under observation at the same period.

The oestrous cycles of these experimental females were characterized by long periods of oestrum; this had a duration in some cases of forty-eight hours in comparison with a length of two to four hours in the normal animal. The cycles were also marked by a great deal of irregularity as can be seen from Figure 8.

The cause of such marked irregularities in the oestrous cycle may be due in part to the trauma incident to transplantation, as shown

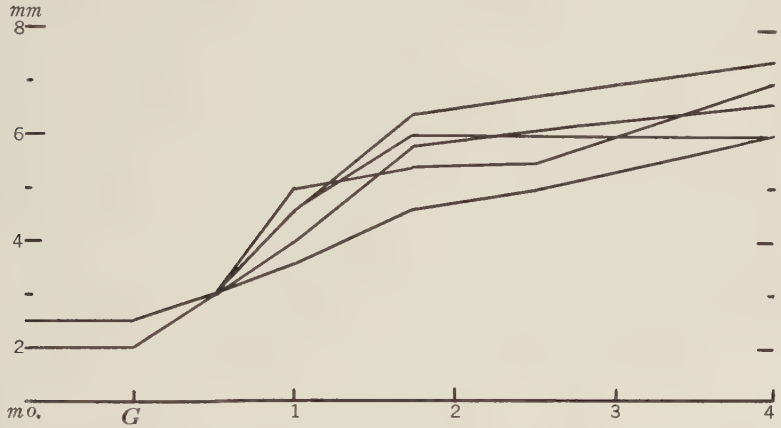

FIG. 7.—Growth response of the nipples of adult prepubertally spayed females to ovarian grafts.

by Wang and Guttmacher (1927) for the rat; they determined that injuries produced surgically on normal ovaries led to a modification of the cycles. However, it is probable that the irregularities depend for the most part upon the influences operative in causing the transplanted ovary to secrete more oestrin.

From this group of observations one may justly conclude that the functional ovarian graft in the kidney of spayed female guinea pigs secretes more oestrin and thus causes a greater mammary hypertrophy than does the normal ovary *in situ* but that it secretes less hormone and thus induces less mammary response than similar grafts in the kidney of castrated and cryptorchid males.

PERIODIC FUNCTION OF THE OVARY IN THE MALE

It is not only an interesting question but a fundamental one to inquire into the causes of greater hormone secretion on the part of an

ovarian graft in a male soma than that by a similar graft in a female soma. Lipschütz (1926, 1927*a*) suggested that ovarian grafts in the male, since they did not produce corpora lutea, were continuously active instead of periodically active as in the female. Due to this continuous activity the male experienced a condition equivalent to one of continuous oestrus and continuous hormone secretion, which was the fundamental cause underlying the greater mammary hyper-

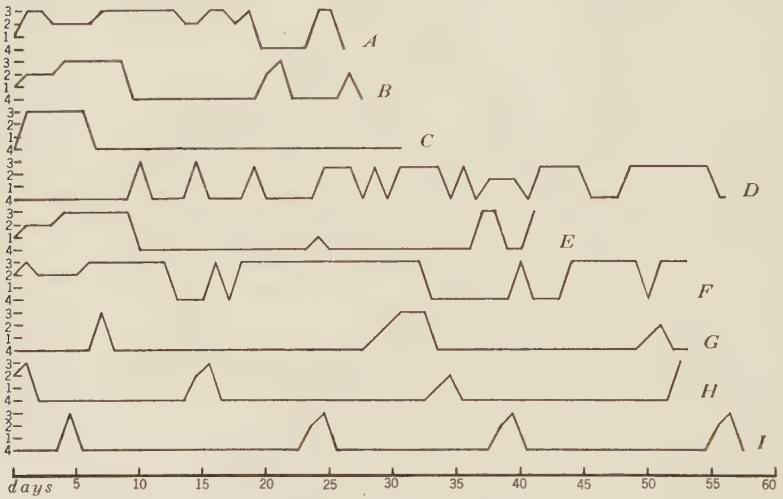

FIG. 8.—Oestrous cycles of spayed females bearing ovarian grafts. *A*, *B*, and *E* show the oestrous cycles at the beginning of activity of the graft. *C*, *D*, *F*, and *G* show the oestrous cycles from 1 to 2 months after the graft first became active. *H* and *I* show the oestrous cycle of unilaterally spayed females. These cycles are normal: (1) nucleated epithelial cells; (2) cornified epithelial cells; (3) nucleated, cornified cells, and leucocytes or cornified cells and leucocytes; (4) leucocytes only or vaginal membrane closed.

trophy. It was of interest to me to learn whether such an explanation was adequate or whether other factors would be involved in the correct explanation.

Two methods suggested themselves as offering a possible solution to the question of the periodicity of ovarian grafts in the male. The first was based upon the changes occurring in endometrial grafts as demonstrated by Markee (1929), and the second was based upon the collection and extraction of excrement of the animals, with tests

for oestrin being conducted by injection of the extracts into spayed female rats. Each method gave suggestive indications but each was far from satisfactory.

Endometrial grafts.—Markee (1929) demonstrated that grafts of small pieces of the uterine mucosa in the anterior chamber of the eye underwent regular changes of vascularity during the oestrous cycle. During dioestrus the mucosa alternately blushed (became more vascular) and blanched, the complete vascular cycle requiring from twelve to twenty-five seconds. During oestrum the graft showed a continuous blush reaction. Markee further showed that ovariotomy abolished the rhythmical changes and led to a continuous "blanch" with disappearance of all "blushes," and that injections of "menformon" (oestrin) returned the continuous blush. Alternate blush and blanch therefore is strong indication of the dioestrous phase of the cycle, whereas continuous blush indicates oestrum.

As a means of attempting to determine whether ovarian grafts were cyclic, small pieces of uterine mucosa were transplanted into the anterior chamber of the eye of male guinea pigs castrated some months earlier, and at the same time an ovary was transplanted into the kidney. Several successful mucosa grafts were obtained in animals with functional ovarian grafts as indicated by mammary responses, but a number of these were complicated and rendered useless by the fact that the cornea became opaque shortly after observations were begun.

Five animals were obtained in which functional ovarian grafts were present and which had successful endometrial grafts, but none of the 5 could be followed for more than a few weeks time. Examination involved daily observations upon the state of the endometrial graft. These 5 animals exhibited a definite blush reaction for several days at a time but there were periods in which distinct blush and blanch periods alternated.

The difficulties involved in this attempt are enormous and the data fragmentary and admittedly lacking in the conclusiveness desired, but so far as they go they give strong indication that the ovarian graft does not maintain a continuous rate of secretion but varies from time to time in the rate of oestrin secreted. This is, of course, the essence of a periodicity that is characteristic of the

ovary in a female animal. It tells nothing concerning the regularity of the process or its degree of approach to the cycle characteristic of the normal female.

Oestrin excretion.—It is known that, in some animals at least, oestrin can be collected from the excrement, particularly the urine of pregnant females. After well-known extraction methods oestrin is easily detected by injection into spayed female rats; cornified smears from the vagina give a positive indication of the presence of oestrin. To determine whether this method possessed any merit for the guinea pig, the total excrement (urine and feces) from a number of pregnant females was collected, dried, and extracted; and, when suspended in olive oil, was injected into spayed rats. The cornified smears proved that it was possible to detect oestrin by such relatively crude technique. With this encouragement an attempt was made to determine whether oestrin elimination was periodic in males bearing functional ovarian grafts.

The total excrement from each of 6 guinea pigs was collected for periods of three days followed by a period of three days without collection. This was made necessary by the fact that the animals were confined in small wire meshed cages suspended above earthenware bowls, and continuous residence in such quarters led to body weight losses and a general decline in good health. The animals occupied the small cages for approximately twenty hours each day, as they were fed in other cages for a period of four hours daily. Each three days of collection, therefore, was followed by a three-day period of rest during which the animals were kept in larger cages with a continuous supply of food. The total excrement from each individual animal for each three days of collection was dried, weighed, powdered, and extracted with benzene continuously for ten hours in a Soxhlet extractor. The benzene was distilled off and the temperature of the residue raised to 110° C. to rid it of benzene and water. The tar-like remains were dissolved in ether and 10 cubic centimeters of olive oil were added to the solution. After evaporation of the ether the material remained in olive oil solution. For assay, $\frac{1}{2}$ cubic centimeter of the oil solution was injected daily for three days into 4 or 5 spayed female rats and vaginal smears were taken twice daily. The 6 guinea pigs from which the excrement was so treated consisted of 2 normal

females, 3 castrated males bearing functional ovarian grafts, and 1 experimental cryptorchid male bearing a functional intra-renal ovarian graft.

TABLE III

THE OCCURRENCE OF OESTROGENIC SUBSTANCES IN THE EXCRETA OF OVARIAN-GRAFT-BEARING ANIMALS

Experimental Cryptorchid Males			Castrate Male	Normal Females			
468	449	407	222	A		B	
				vaginal smears		vaginal smears	
+	+	+	−	oestrum	+	oestrum	+
+	−	+	+		−		−
−	−	−	−		−		−
				oestrum		oestrum	
−	−	−	−		−		−
−	−	+	+		−		−
−	−	+?	−	oestrum	−	oestrum	+
+	−	−	+		−		−

Excreta (urine and feces) collected on alternate 3-day periods. Blank spaces represent the periods during which no collection was made. Plus (+) indicates the presence of oestrogenic substances in that sample as shown by the positive reaction of all of the assay animals. Minus (−) refers to a negative reaction of all of the test animals on injection of excrement extracts into spayed females.

The results obtained from an assay of the excrement of this group of 6 animals is given in brief form in Table III. The method proved sufficiently delicate to detect oestrin in the excrement from the normal females during the period of oestrum but gave no indication of its presence during the dioestrous period; these periods were of

course easily determined in the normal females by the vaginal smears. The excrement collected from the males bearing functional ovarian grafts was positive during some of the three-day periods but negative during others. In some cases positive reactions were given for two succeeding three-day periods.

These observations are interpreted to mean that the ovarian grafts in the castrated and experimental cryptorchid males were producing sufficient oestrin to be detected by injecting the extracts from the total excrement into spayed female rats. The amount of oestrin excreted within a period of three days was not constant but varied between such extremes as easily detectable amounts and amounts that were not detectable by the methods employed. The data obtained do not serve to indicate the regularity of this cyclic event nor the total time between two most active periods of excretion, because instead of showing results for a continuous period sufficiently long to encompass a normal cycle of the female it covers only periods of three days separated by other three-day periods for which no data are available.

Both the endometrial graft observations and those made from the collection of excrement, therefore, indicate very strongly that the period of oestrin secretion by these ovarian grafts in treated males varies rather than remains constant. The fact that such periods of more abundant secretion occur is good evidence that cyclic phenomena characterize the activity of these grafts, but it tells us little concerning the regularity of these periods or how nearly comparable they are to the similar phenomenon that is the regular occurrence in the normal ovary of the female. The observations do not support the suggestions of Lipschütz (1927*a*) that cyclic phenomena are absent in ovarian grafts in males, hence some other explanation must apparently be adopted to account for the difference in mammary responses to ovarian grafts in spayed females and castrated males.

HYPOPHYSIAL DIMORPHISM

The difference between the responses of the mammary apparatus in the castrated male and the spayed female guinea pig in which ovarian intra-renal grafts are the responsible agents would seem to have been clearly demonstrated, by the observations recorded in

preceding portions of this discussion, to be dependent upon the amounts of oestrin liberated by the grafts. The reactivity of the male and female mammary glands to injections of oestrin was shown to be essentially identical. The key to the difference therefore rests with those forces which govern the output of oestrin from the grafts. The suggestion of Lipschütz (1926, 1927*a*), that the difference depends upon the lack of periodicity in the ovarian graft in the male does not receive substantiation from the material which is here reported.

The most plausible explanation that suggests itself involves the well-known stimulant to gonad activity—the hypophysis. This gland which controls the activity of the ovary must therefore exhibit a physiological sex-dimorphism which is responsible for the greater oestrin production of ovarian grafts in the male than in the female organism. That such a physiological sex-dimorphism is operative under normal conditions is indicated by the fact that hypophyses from male animals are more potent in stimulation of the gonads of prepubertal animals than are those of females (Evans and Simpson, 1929). Is this a condition imposed upon the male hypophysis by secretions from the testis and a lower capacity imposed upon the female hypophysis through secretions from the ovary, or is it a genetic character arising without any relation to secretions from the gonads as the animal develops to maturity? If it is a condition—a physiological reaction—that is in any way conditioned by the development of the respective sex hormones, early gonadectomies accompanied by ovarian transplantation should lead to similar responses on the part of the mammary apparatus in both sexes. If, on the other hand, it is a genetic character the same differences in mammary response in the two sexes should follow, even after very early gonadectomies, as followed transplantations in adult animals. To learn the relative responses in the young animals a group of brother-sister animals was utilized.

Four pairs of brother-sister guinea pigs were selected and the male was castrated a few days after birth, receiving at the same time one ovary from its sister as an intra-renal graft. The male and female thus possessed one ovary each, but differed in that the ovary in the male was a graft, whereas that of the female was normal (had

not been disturbed). Measurements of the nipples were recorded for a period of six months, and the trend in nipple growth is recorded in Figure 9.

At the termination of the experiment the nipple height of all the treated males was considerably greater than the nipple height of their sisters carrying the corresponding but undisturbed ovary.

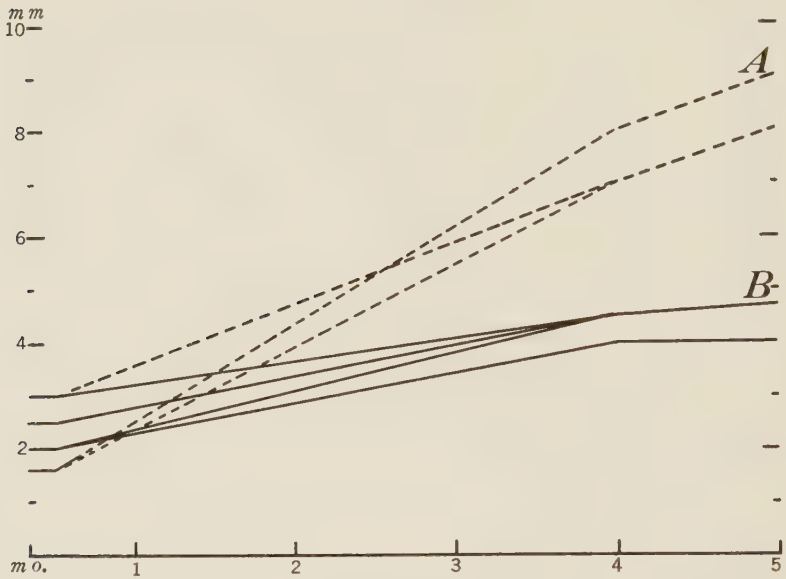

FIG. 9.—Growth response of the nipples of young prepubertally castrated males (*A*) to ovarian grafts made before puberty. The controls were unilaterally spayed sisters (*B*) of the males, and donors of their grafts. The months refer to the age of the animals.

Thus the nipple height of the males was from 8 to 9 millimeters whereas the nipple height of the females was from 4 to 5 millimeters.

In order to eliminate a factor which might have affected the result due to the undisturbed ovary of the female, in contrast to the grafted ovary of the male, a second set of animals was utilized in which each sex carried only a grafted ovary. Five pairs of brother-sister animals were utilized in which the male was castrated before the age of fifteen days and received at the same time one ovary from its sister. At the same time also the second ovary was removed from its normal site and placed in the kidney of the female donor (auto-

graft). The 2 animals were therefore equal in every respect with the exception of their genetic constitution.

The growth of the nipples of these two groups of animals is expressed in Figure 10. Nipple growth began about the normal age of puberty and in some cases was apparent considerably earlier in the male than in the female. Furthermore, the growth of the male nipples was more rapid than that of the females and when observations were discontinued were much larger than those of the females.

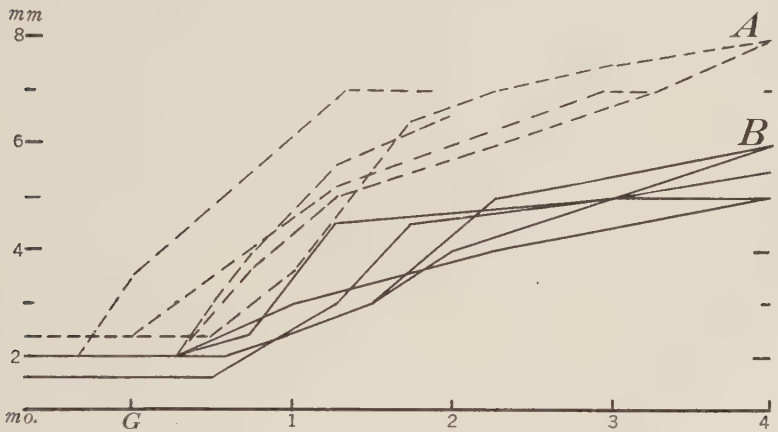

FIG. 10.—Growth response of the nipples of young prepubertally gonadectomized males (*A*) and females (*B*) to ovarian grafts made before puberty. The months refer to the age of the animals.

This result appears to be open to but one interpretation—namely, since the two ovaries from the female donor were transplanted at the same time, in the same manner, and to similar positions, their inherent potentialities should have been equal. Differences that they may exhibit must lie outside their own domain and be conditioned by those forces which become operative upon them. Since the hypophysis is known to govern the activity of the ovary and control its oestrin production, and since it has been shown earlier in this paper that the potential reactivity of the male and female mammary glands to oestrin is the same, the greater oestrin secretion by the graft in the male must be attributed to a greater stimulation issuing from the hypophysis. All influences emanating from the sex glands

were removed before they could have become sufficiently potent to condition the responses of other organs of the animal, such as the hypophysis. The explanation must rest, therefore, upon a physiological sexual-dimorphism exhibited by the hypophyses, with the capabilities for ovarian stimulation being greater in the hypophysis of the male than in the female. It would therefore seem to be a sex-limited genetic character.

LACTATION

Among the large group of treated males bearing active ovarian grafts, 2 animals only gave any evidence of lactation. One of these was a castrated male, the other an experimental cryptorchid male. In each case nipple height was below the maximum attained in a number of other animals. The amount of milk expressible at a given time consisted of a few drops only, and the flow continued but for a few days. The reason for the discrepancy between results reported here and those of Steinach (1913, 1916), Lipschütz (1926), Sand (1922*c*), Athias (1915, 1916) and Pettinari (1925) is not yet clear.

It was thought possible that for some reason male guinea pigs with ovarian grafts were incapable of milk secretion. A study of the ovarian grafts revealed the absence of corpora lutea, and the mammary gland tissue in sections resembled that of a female in an early stage of pregnancy rather than one near parturition. Since many authors have ascribed the development of a functional mammary gland to the corpus luteum and since it is well known that administration of anterior pituitary extracts induces corpus luteum formation and stimulates milk secretion in the adult female, the effects of injection of such substances into the graft-bearing males were investigated.

Three castrated males and 3 experimental cryptorchid males, each bearing a functional ovarian graft, were injected twice daily for a few days with an aqueous pituitary extract (an alkaline extract of sheep pituitary prepared by Dr. O. Kamm of Parke Davis Co.). Whereas there was no measurable increase in the nipple height from this treatment, these males secreted milk (preceded by colostrum) within fifty hours from the time of the first injection.

When the sections of the ovarian grafts from these pituitary ex-

tract injected males were studied, well-developed corpora lutea were found.

In order to determine whether the presence of a corpus luteum in the ovarian graft was required for lactation, 3 treated male animals with large nipples were injected with the aqueous pituitary extract eight hours after removal of the kidneys in which the ovarian graft was located.

Despite the absence of any definite corpus luteum tissue in the graft that was removed before the pituitary extract injection, these males all lactated as in the cases previously cited.

In order to eliminate all possible sources of corpus luteum secretion, as from diffuse tissue possibly giving secretions equivalent to a well-formed corpus, experimental cryptorchid males which had not received ovarian implants were utilized. These males, with testes confined in the abdomen, were injected with oestrin known to be free from the lutein hormones until their nipple development was as great as that of a pregnant female (a height of 9–11 millimeters). The animals were then injected with the pituitary extract, and milk secretion began within forty-eight hours.

The results of this group of experiments clearly demonstrate that the hypertrophied mammary apparatus of the treated male guinea pig is capable of lactation if only anterior pituitary substances are administered.[3] They demonstrate further that lactation can be induced in the male mammary glands without lutein secretions ever having been involved in the stimulation. The results, however, do not indicate that the corpus luteum secretions are useless in ordinary lactation in normal females.

The absence of lactation in practically all males bearing functional ovarian grafts and well-developed mammae may be due to the lack of anterior pituitary secretions, the production of which is inhibited by oestrin produced by the graft. The degeneration of such a graft followed by a decrease in oestrin secretion would cause a sudden increase in anterior pituitary hormone available, which when present in sufficient quantities has been shown to result in lactation in such animals. An analysis of this suggestion is in progress.

[3] This question will be treated at greater length in a subsequent publication in conjunction with Dr. W. O. Nelson.

EFFECT OF OVARIAN GRAFTS ON TESTIS HORMONE SECRETION

The enormous growth of the nipples of both the castrated males and the experimental cryptorchid males guarantees that relatively large amounts of oestrin were being produced by the ovarian grafts. Several writers who have injected the normal male with oestrin have reported an "anti-masculine" effect of some character, involving such structures as the gonads and accessory reproductive organs (see Laqueur, Borchardt, Dingemasse, and De Jongh, 1928; Steinach and Kun, 1926 and 1931; Fellner, 1921; Moore, 1930*a;* and Moore and Price, 1932). Some of these authors have attributed the result to an antagonism between the sex hormones, but Moore and Price have assembled a great weight of evidence pointing to a reciprocal influence between the sex glands and the hypophysis as the probable explanation. Thus, they maintain that introduction of oestrin, rather than having a direct effect upon the testes and accessory organs, acts to interfere with the hypophysis in such a manner that the hypophysial secretions necessary to maintain gonad function are diminished, and the injury to the male system would thus be due only to a deficient amount of these necessary substances. If this explanation is correct, one would expect that the presence of the oestrin secreted by the ovarian grafts in experimental cryptorchid males would in some way modify the secretion of testis hormones by the abdominal testes.

In order to determine whether there was evidence for diminished testis hormone secretion, a study was made of the amount of the ejaculate each week as determined by the electric ejaculation test (see Moore and Gallagher, 1930).

The ejaculate was obtained each week from a group of 21 experimental cryptorchid males carrying functional ovarian grafts. These ejaculates were compared with those collected from a group of 18 normal males. They were further compared with ejaculates from a group of 27 experimental cryptorchid males, 19 of which had received ovarian transplants that failed to become incorporated, and 8 of which had never received ovarian transplants. The record of these observations is given in Figure 11. The first ejaculate obtained from each animal was discarded in order that those considered

might more adequately express the rate of secretion on the part of the prostate and seminal vesicles which are the principal source of the material composing the ejaculate. The first ejaculate rather than indicating the amount of secretion for the previous week more often is larger in amount from the factor of storage of the secretions from several days longer than one week. The average of all ejaculates from each animal collected, subsequent to the first, would therefore

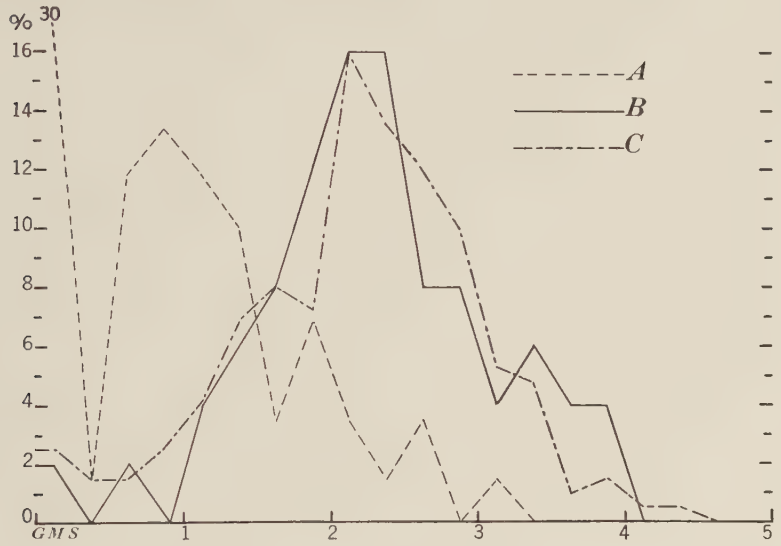

FIG. 11.—Distribution curves of the ejaculate weights of: *A*, cryptorchid males bearing ovarian grafts (59 weights); *B*, normal males (50 weights); *C*, cryptorchid males (191 weights).

establish quite satisfactorily the average rate of secretion of the accessory organs. This would in turn serve as an index of the rate of testis hormone secretion, since the activity of these organs is under the direct control of the testis.

Figure 11 shows the ejaculates plotted in a distribution curve for the three groups. The curve of the ejaculate distribution from the ovarian-grafted cryptorchid males is based upon 191 points. The curves for both the normal males and the experimental cryptorchid males are almost identical, which shows that the abdominal testes entirely devoid of germinal tissue are secreting as much testis hor-

mone as do the testes of the normal males. The secretion of that portion of the testis hormone, therefore, which controls the accessory reproductive organs remains unchanged in the degenerate abdominal testicle. On the other hand, the amount of ejaculate produced by the experimental cryptorchid males bearing functional ovarian grafts is much below that of the control groups. This is interpreted to mean that the oestrin secretion of the ovarian graft serves effectively to inhibit the hypophysis in its activity and to reduce the quantity of hypophysial secretions available to stimulate the secretory activity of the abdominal testes. As a result of the diminished testis hormone production a smaller amount of ejaculate is obtained after electrical stimulation.

In order to determine more absolutely, if possible, whether oestrin does have such an effect in the male guinea pig, 16 adult males were rendered experimentally cryptorchid, were divided into two groups of 8, and ejaculations were collected each week for a period of eight weeks. Beginning at the ninth week one group received daily injections of 1 rat unit of oestrin for a period of four weeks; the second group served as the control. The daily injection of oestrin in the experimental group was then increased to 2 rat units for a period of two weeks, then to 4 rat units for two weeks, and finally a daily injection of $12\frac{1}{2}$ rat units was given for four weeks. The average weight of the ejaculates from all animals of each group is plotted in Figure 12 and covers the eight weeks preceding injection and the consecutive weeks during which the different dosages of oestrin were administered.

An examination of this graph reveals the fact that the ejaculate curve from the group of animals receiving a daily injection of 1 rat unit of oestrin diverges from that of the uninjected ones to a detectable degree. As the dosage was increased to 2, 4, and 12 rat units daily, the average weight of the ejaculate of the injected group decreased, whereas that of the control group remained essentially constant. This result shows that oestrin injection does cause a decrease in the secretory activity of the accessory reproductive organs, which in turn is to be explained only on the basis of a lowered hormone secretion by the cryptorchid testes. This lowered testis secretion is to be explained on a diminished available amount of hypophysial

secretions caused by the activity of oestrin, which is injurious to the hypophysis. The results, therefore, confirm the contentions of Moore and Price that a reciprocal physiological influence is operative between the gonads and the hypophysis. Their data which formed the basis for such an explanation were obtained almost entirely from the rat, and my own observations on the guinea pig offer strong confirmative evidence that the explanation is the correct one.

The conclusions drawn from material in this section are that an ovarian graft causes a diminution in the function of the accessory organs of the male guinea pig, and that the influence is probably due to the secretion of oestrin. Purified oestrin samples given in sub-

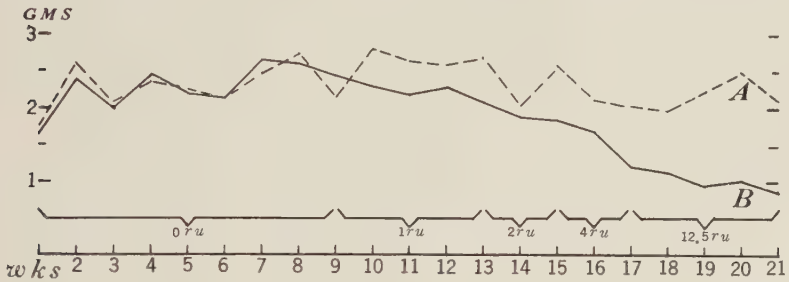

FIG. 12.—Average ejaculate weight of experimental cryptorchid males. *A*, controls (8 animals) uninjected. *B*, treated males (8 animals)—no injection for first eight weeks after testis elevation. Succeeding four weeks one rat unit of oestrin injected daily; succeeding two weeks 2 rat units of oestrin injected daily; final four weeks 12.5 units of oestrin daily.

cutaneous dosages of known strength were found to have an influence entirely similar to that exerted by the functional grafts. The diminished secretion of these accessory structures is due to a diminished secretion of testis hormone. The manner in which oestrin causes a lowered testis hormone production involves an interference with hypophysial function.

BEHAVIOR OF OVARIAN GRAFTED MALES

One of the many interesting phases of the problem of the effect of sex hormones upon the organism is that reputed to affect, or condition, behavior. A systematic and careful study of this phase has not been attempted in this investigation, but in numerous instances casual observations made on the group of animals with which I have

worked lead me to offer them only as observations without attempting a careful analysis or extensive interpretation.

Normal males kept in groups of a dozen or more in large pens fight considerably. It is also true that females will fight among themselves but not to the extent exhibited by males. Experimental cryptorchid males behave entirely as normal males. Prepubertally gonadectomized males and females seldom fight. Pregnant females, or those suckling young, drive away other animals of both sexes. Males may mount one another in an attempted coitus and may often experience an ejaculation, but a penchant of one female for another has not been noticed.

Experimental cryptorchid males bearing functional ovarian grafts, as testified by enlarged mammae, fight among themselves and mount in typical male fashion. Their mating reactions with females are as typical as those of normal males.

In the course of the observations on lactation, young suckling animals a few days after birth were taken from their mothers and confined with the lactating males in small pens. Although driven by hunger to make repeated and vigorous attempts to suck, these young animals were consistently and vigorously repelled by the male, castrated a few days after birth and carrying a functional ovarian graft for months. The animal was lactating, and, when it was forcibly held, the young did nurse. However, not even passive resignation was exhibited after forceful restraint for several days during which the young nursed. Among the large number of castrated males with active ovarian grafts that have served as a basis for this study, none has exhibited any tendencies that could be interpreted as showing the possession of maternal instincts. The behavior reactions of these male animals were entirely characteristic of males though they had lacked testes since before puberty. Such observations are in disagreement with those of Steinach (1916) and Sand (1918); they substantiate similar observations of Moore (1921*b*).

HISTOLOGICAL CHARACTER OF OVARIAN GRAFTS

A remarkable agreement appears to exist in the several accounts of the structure of ovarian grafts in male animals. In general one finds considerable follicular atresia, a tendency toward cyst forma-

tion, and an absence of well-defined corpora lutea. Voss (1926) notes a striking tendency toward calcification in the connective tissue.

The ovarian grafts examined by me have exhibited practically the entire range of possibilities described by others. The simple technique of Bouin fixation and haematoxylin and eosin staining has been employed, and all cases involved the tissue surrounding the graft—either kidney or testis tissue—depending upon the locus of the graft. The implantation sites of 75 positive cases and 50 negative cases (those in which mammary responses were absent) have been sectioned serially. Since the reactions of grafted ovaries have been treated so often in previous writings, and since my own observations introduce no particularly striking new facts, a brief mention only of the conditions found will be attempted.

The one outstanding difference in ovarian grafts recovered from males (castrated or experimental cryptorchids) and those recovered from females is the presence of well-formed corpora lutea in the latter group. As mentioned in a previous section, those males receiving injections of pituitary extract produced typical corpora. But in addition to this outstanding difference it appears to be a general rule that grafts from the males contained more numerous and larger Graafian follicles than did those from females. The number of small (primary) follicles, as a rule, was not great, but this may not be surprising in view of the fact that a germinal epithelium was absent in all cases, due to the mode of incorporation of the graft in the host tissue. Furthermore, most of the ovarian grafts removed for section had persisted in the hosts for periods ranging usually from twelve to eighteen months.

All grafts recovered from animals that had given positive mammary response contained at least a few normal follicles, whereas no graft studied from a case in which mammary response was negative contained normal follicles.

Ovarian grafts recovered from experimental cryptorchid males exhibited no marked differences from those recovered from castrated males. Each group revealed normal follicles in different stages of maturity that contained normal-appearing ovocytes. The fact that the experimental cryptorchid males were producing testis hormone in amounts equivalent to that produced in normal males and yet

showed no modification in the response of the grafted ovary is important.

It follows from these observations, therefore, that the normal secretion of testis hormone in experimental cryptorchid male animals is without demonstrable effect upon the reactions of the ovarian tissue in either an anatomical or a physiological sense.

DISCUSSION

The problems involved in the general question of sex hormone antagonism as it has been introduced by Steinach, modified by Sand, Lipschütz, and others, and criticized by Moore, and Moore and Price, are broad and complex.

It is no longer to be doubted that the original contentions of Steinach, that heterosexual gonad grafts could not be successfully made in a normal host of the opposite sex, were incorrect. This has been disproved adequately by the publications of Sand, of Lipschütz and his co-workers and students, and of Moore, and is further disproved by the observations presented here and by many casual observations of other workers. Certain of the contentions in Sand's modification of Steinach's antagonism conception are wrong, but others, at least with certain modifications, will stand. Thus Sand's failure to obtain persistence of an ovarian graft in a normal male host in localities other than the testis is not only unimpressive but thoroughly misleading in its implications. Lipschütz and his students have demonstrated many times that ovaries transplanted into the kidney of a normal male will become incorporated successfully and persist for a long period of time. This is adequately confirmed by the material herein presented. That such ovarian grafts seldom exercise their potential hormone-secreting capacities is another question and will be dealt with below. The testicle, therefore, does not present any particular merits as a locus for ovarian grafts excepting for its accessibility, size, and excellent vascular bed. Transplantation of ovaries into the kidney or other sites is just as successful as transplantation into the testicle.

The contentions of Sand, involved in his familiar "atreptical immunity" hypothesis (1918, 1919, 1922*a*, *b*, *c*, and 1923), when changed to fit more recent contributions to our knowledge regarding

sex gland activity has considerable merit. Persistence and function, rather than being dependent upon the capture by the testis of certain "food substances in the circulating blood stream" thus making them available to an intra-testicular ovarian graft, can be understood to mean the dependence of the ovarian grafts upon certain substances produced by the hypophysis for their persistence and function. Not only does this idea stand confirmed, but it indicates one of the first signs of recognition of the dependence of the gonads for persistence and function upon substances produced elsewhere in the organism. Instead of a nutritive principle in the generally accepted sense, however, it must be recognized as a hormone stimulation, the secretions responsible for the action coming from the hypophysis.

A further confusing contention in the general problems involved is that sex hormones produced in the two sexes are antagonistic, but the degree or character of such an antagonism has not been well defined. Steinach's earlier conception of the direct inhibition or antagonistic effect of a hormone upon the heterologous end organs is no longer tenable if due respect is paid to the observations presented by Moore and Price, and to those contributed in this publication. Thus, Moore and Price have adequately demonstrated that the accessory reproductive organs of the castrated rat will respond to a given dosage of testis hormone in like manner when this is administered alone, or accompanied by large potent dosages of oestrin. Similarly, the mammary gland of the guinea pig will respond to minimal effective doses of oestrin either in the presence, or absence, of large quantities of testis hormone.

There are other difficulties and somewhat confusing conditions, however, for, as Lipschütz has demonstrated and the present work confirms, the presence of a normal testicle, and of an abdominal testicle that is devoid of germinal epithelium, exerts different effects upon the activity of an ovarian graft. The normal testicle, though permitting the successful incorporation and persistence of an ovarian graft, will usually prevent it from carrying on its endocrine secretion. The abdominal testicle which lacks an active germinal epithelium, however, not only permits the incorporation of an ovarian graft, but also permits as great an endocrine secretion by

the graft as ocurs in a castrated male. Each of these two types of testes are accompanied by the same degree of activity on the part of the male accessory reproductive organs—seminal vesicle, prostate gland, etc.—hence, in the usual sense, they can be said to secrete equivalent amounts of testis hormone. This introduces the question of "multiplicity" of the testis hormone. Is the internal secretion of the testicle one substance, or does it consist of more than one substance?

The testis hormone, entire or in part, has been extracted from the fresh testicle of the bull and other mammals, and from human male urine (McGee, 1921; Gallagher and Koch, 1929; Funk, Harrow, and Lejwa, 1930; Loewe and Voss, 1929; Freud, de Fremery, and Laqueur, 1931), and a crystalline product from urine has been obtained by Butenandt (1931). The chief methods of assay have utilized the capon comb method, and whereas the material stimulates the capon comb growth without question, it is still to be demonstrated whether such substances as have this power include all the substances that are normally secreted by the mammalian testis.

In a co-operative study of the testis hormone in this University, mammals have been utilized extensively in testing the effectiveness of the substances prepared by Gallagher and Koch from bull testis extract. The reactions in the mammal to these substances have been studied by Moore and his co-workers. They have attempted an exhaustive study of the effects of castration in rats and guinea pigs particularly, in order to determine as nearly as possible all the effects that follow complete elimination of the testis. As a result of these studies approximately nine or ten hormonal deficiency effects have been described which encompass a variety of hormonal functions. These have included the effects upon the life of unshed epididymal spermatozoa in the guinea pig (spermatozoan motility test) and the physiological activities of the accessory reproductive organs as determined by inducing an ejaculation in the guinea pig by electrical stimulation on the head (electric ejaculation test). They have included also a thorough investigation of the accessory reproductive organs in the rat —seminal vesicles, prostate gland, Cowper's gland, vas deferens, epididymis, etc.—by cytological methods.

Moore has repeatedly stressed the necessity of learning all the deficiency effects following castration before concluding finally that a given preparation of hormone contains all the substances normally secreted into the blood stream by the testis.

The indication that the preparations of Gallagher and Koch contained the complete hormone or hormones was given by the fact that their injection into castrated animals served to ward off all detectable effects following testis ablation.

An effect of castration more recently being emphasized, and not yet entirely clear, is the response of the hypophysis both anatomically and physiologically. It was shown by Engle (1929), Evans and Simpson (1929), and others that castration is followed by an increased potency of the hypophysis to stimulate sex gland activities upon its implantation into immature animals, and it has been noted by many writers that cellular changes in the hypophysis accompany such an increase in stimulating powers, especially in the rat ("castration cells"). There is some evidence that these hypophysial responses to testis hormone, or its absence, depend upon substances not involved in the regulation of the accessory organs. Thus Martins and Rocha (1931*b*) claim that certain extracts from the testicle will prevent the development of castration changes in the hypophysis of the rat and yet be incapable of exercising a hormonal control over the accessory organs. Still other extracts that adequately maintain the normal condition of the accessory organs do not prevent the appearance of castration cells. Furthermore, experimental cryptorchid animals possessing entirely normal accessory reproductive organs show the typical castration cells. The substance having the regulatory control over the hypophysis is thus believed to have its source of origin in the germinal epithelium, whereas it is well known that the accessory reproductive organs are maintained indefinitely in a normal state in the complete absence of a germinal epithelium (experimental cryptorchid guinea pigs).

The work of Lipschütz, and that reported here, clearly demonstrates a different response on the part of an ovarian graft in the presence of testes with an active germinal epithelium and those without it. But it is not entirely clear from this and other work whether the differences do not involve a quantitative factor in the

reciprocal gonad-hypophysial interrelationship. If there are two separate components to the testis hormone, one exercising a control over the hypophysis, the other over the accessory reproductive organs, it would appear that they are obtained by the same methods of extraction and purification. The preparations of Koch and Gallagher have been shown to be entirely adequate in maintaining the normal conditions of the accessory reproductive organs in the castrate mammal (Moore and Gallagher, 1930; Moore, Hughes, and Gallagher, 1930; Moore, Price, and Gallagher, 1930; Vatna, 1930; and Heller, 1932); and, after the injections of Gallagher's preparations, and others prepared in a similar fashion, into castrate rats, Reese and McQueen-Williams (1932) claim that castration cells are also abolished. These results indicate either that a single substance is involved in the hypophysial and accessory reproductive gland control or that if more than one is involved all are present in these extracts.

Assuming therefore that the complete testis hormone is contained in the testis extract preparations of Gallagher and Koch, one must immediately grant the contention that no antagonism exists between these and oestrin. The delicate response of the mammary glands of the guinea pig, as herein described, to amounts of oestrin of the order of two-tenths of a rat unit daily is clearly demonstrated. When this quantity is administered, along with sufficient quantities of testis hormone to produce coagulable ejaculates in the castrated males, and yet produces the same response in the mammary glands as when injected alone, there appears to be no cause for considering an antagonism to exist between the two substances.

Such basic information in regard to mammary response, and this applies to either male or female mammary glands, permits an approach to consideration of the response of these end organs to ovarian transplantation.

In the normal male guinea pig ovarian grafts persist but only seldom do they evoke a response in the mammary glands. This is not due to the presence of the testicle or its secretions, except indirectly, but only to the fact that the graft is not secreting hormones; for, when oestrin is injected into the normal male, mammary response follows. It is obvious, therefore, that the ovarian graft in the normal

male does not secrete oestrin. That it is capable of so doing is easily demonstrated either by removal of the testicle or by elevating it into the abdomen. It appears reasonable to believe that the lack of oestrin secretion is due merely to the insufficient quantity (possibly quality?) of those substances that force an ovary to secrete—namely, hypophysial sex-stimulating substances. This hypophysial sex-stimulating substance can be increased in a male in at least three ways: (1) It is increased by removal of the testis. This is conceived to act in two possible ways. In the first place, the testicle probably utilizes the substance in some manner in its general metabolism. This is indicated by the fact that in administration of the sex-stimulating substance the response is at least roughly proportional to the amount administered; nothing is known, however, in regard to its mode of action. In the second place, castration removes the suppressing influence of the sex hormones on the hypophysis. That the hypophysis is suppressed by the sex hormones and is more active in their absence is proved by gonadectomy (Engle, 1929; Evans and Simpson, 1929) and by injection of the sex hormones (Meyer, Leonard, Hisaw, and Martin, 1930; Moore and Price, 1932; Spencer, Gustavson, and D'Amour, 1931; Wade and Doisy, 1931). (2) The hypophysial sex-stimulating substance can also be increased by rendering the animal an experimental cryptorchid. Hypophyses of experimental cryptorchid males are intermediate in their activity between those from normal males and the more active ones from castrated males (Evans and Simpson, 1929; Kallas, 1930; Martins and Rocha, 1931). (3) Finally, the sex-stimulating substance can be increased in the animal by direct injection. The first two methods lead to an immediate response of the mammary glands in the guinea pig bearing an ovarian graft, as shown by both Lipschütz and myself. The third method, that of direct injection, has also been shown by Engle (1928), in the male rat bearing an ovarian graft, to induce mammary hypertrophy while the normal testes were present and active. In the rat it was the mammary gland tissue which responded and not the nipple, since male rats do not possess rudimentary nipples.

It can be readily understood, therefore, that the conditions which prevent the ovarian graft from exercising its potential influence upon

the mammary gland of the normal male are believed to be due merely to the fact that the hypophysial substances are not available to stimulate ovarian graft activity because of the activity of the testicle. There is a sufficient amount of secretion for the activity of the testes but not for both testes and ovary. It is not clear why the testis can maintain its activity and the ovary only incompletely so. Threshold differences of the ovarian and testicular tissues might be a factor, and it is also conceivable that qualitatively different substances are involved. The presence of the testicle is antagonistic but only by virtue of its influence upon the hypophysis, and not from any inactivation of oestrin or any inhibitory influence upon the mammary gland.

It is an interesting fact that the mammary gland responses to ovarian grafts in castrated males and experimental cryptorchid males are greater than those in spayed females with ovarian grafts or in those of the normal female. It must be remembered that this difference is not due to a difference in the capacity of the mammary glands of the two sexes to respond to oestrin. Their equipotentiality in the two sexes is clearly demonstrated by the experiments here reported. The difference depends here again entirely upon the actual secretion of oestrin. The fundamental question, therefore, is why ovarian tissue in the male soma secretes more actively than that in the female soma. Again the answer appears to be obscured by lack of knowledge concerning the function of the hypophysis and the interactions between it and the sex hormones. Ovaries respond to hypophysial secretions and produce more oestrin but appear to be modified in their response by the formation of corpora lutea. There is general agreement that corpora lutea are formed in ovarian grafts in the female but not in the male. Is this due to quantitative differences in the male and female hypophysial secretions or to qualitative differences? It is impossible at the present time to give the final answer, but the investigations of many workers and observations of my own make it seem highly probable that a genetic difference exists in the hypophysis of male and female animals. Hypophyses of normal males are more potent in stimulating precocious sexual maturity than those of normal females; and those of castrate males are more potent than those of spayed females (Evans and

Simpson, 1929). Furthermore, as my experiments show, of two ovaries from the same immature female transplanted, one into the kidney of a castrated brother, the other into her own kidney, at the age of ten days (therefore each animal was without gonads except the ovary graft), the graft in the male soma invariably secreted more oestrin than the one in the female soma. This fact, however, does not demonstrate whether the cause lies in a quantitative or qualitative difference in the two hypophyses. The gonad-hypophysial interactions are complicated in the female, not only by the apparent quantitative difference (perhaps qualitative) in its secretory activity, but also by the formation of lutein tissue. Secretions from the latter appear, from the work of others, to influence the response of the ovarian tissue and in the second case they may also affect the activity of the hypophysis in a manner different from that of oestrin. The solution of the problem of the differences in ovarian response in the male and female soma must await further study.

The question of the periodicity in function of ovarian grafts in the male is not satisfactorily disposed of by my observations. Periodicity in ovarian function in the female is an outstanding characteristic. The mechanism involved in the regulation of this periodicity as explained by Moore, 1930*a;* Brouha and Simmonet, 1930; Moore and Price, 1932, are the reciprocal interactions between the ovary and hypophysis. Thus Moore, and Moore and Price visualize the cycle, starting with the ovary, as first dependent upon a stimulation of the ovary by secretions from the hypophysis. This leads to follicular ripening and an outpouring of oestrin. Oestrin then brings on the oestral response (vaginal cornified smears and mating), and the concentration of oestrin serves effectively to arrest hypophysial secretion. The hypophysis therefore exhibits a low state of secretion during the time oestrin exists in a high concentration in the body, and it is during this time that the ovary is inactive in follicular development. When the oestrin concentration is reduced in the body, through excretion, the hypophysial activity occurs, thus inducing a new cycle. It is well understood, of course, that many conditions can operate to disturb this delicate controlling mechanism and lead to irregularities or total suppression of the cycle.

Granting for the moment the possible correctness of the explana-

tion of the mechanism, it does not follow that the male hypophysis would fit into the scheme as adequately as does the female hypophysis. The discussion above takes into consideration some of the evidence that the hypophyses of the two sexes are fundamentally different in some phases of their physiology. That the male hypophysis is affected by oestrin is clearly indicated by the work of Moore and Price, 1932; Spencer, Gustavson, and D'Amour, 1931; and Wade and Doisy, 1931; but these experiments do not prove that the threshold of effect is the same as that for the female hypophysis.

The evidence presented in this paper on the activity of endometrial grafts in the anterior chamber of the eye of males bearing ovarian grafts, and that from the excretion of oestrin by males with ovarian grafts, indicates strongly a periodicity of the ovarian graft in the male. Unfortunately, however, it was almost impossible to form any judgment in regard to the regularity of the periodicity by the experimental method involved. A final solution of this question also must await further work. So far as they go the results point definitely to a periodic function on the part of the ovarian graft in males.

The problem of lactation will be considered more adequately in another paper. It is sufficient for the purposes here to re-emphasize the almost total absence of lactation in mammary glands in the male bearing ovarian grafts, despite the much greater activity than exists in the normal non-lactating female. The proper stimulus for lactation is not provided, but this cannot be dispensed with by assuming the cause to rest wholly with the absence of lutein tissue. It should be recalled that castrated males injected with lutein-free oestrin and subsequently with alkaline extracts of pituitary secrete copiously despite the fact that ovarian tissue had never been present in the animal. Again, this is not an argument against some influence from lutein tissue on lactation in the normal female, but it brings into question the absolute necessity of lutein secretions for lactation.

The final point for consideration and emphasis is the effect of the ovarian graft on the secretion of testis hormone in experimental cryptorchid males. That the oestrin secreted by the ovarian graft, or oestrin introduced by injection, does lessen the secretion of testis hormone in such animals is scarcely to be doubted if close attention

is given to the evidence presented. These observations on the effect of oestrin in the male guinea pig are entirely in line with the observations of Moore and Price on the rat. They are readily interpreted, as these authors have pointed out, on the basis of an inhibitory action of oestrin on the hypophysis, which reduces the available amount of the sex-stimulating substances for testis activity. The correctness or inadequacy of this explanation must await the collection of further evidence.

SUMMARY AND CONCLUSIONS

1. The mammary glands of male and female guinea pigs are somatic, non-sex-dimorphic, equipotential characters. They respond equally in the two sexes to the injection of oestrin.

2. There is no antagonism between oestrin and testis hormone. Oestrin alone, or injected along with effective doses of testis hormone, brings forth similar quantitative responses in the mammary glands of both sexes.

3. Ovary grafts in normal males persist but do not secrete oestrin. Abdominal elevation of the testes or castration permit the graft to secrete oestrin with mammary response greater than in normal or ovarian-grafted females.

4. The explanation of differences in mammary responses in males and females does not involve direct interaction of sex hormones or hormone antagonism. It involves the reciprocal gonad-hypophysial interactions.

5. Ovary grafts in experimental cryptorchid or castrated males appear to be periodic in function; this is indicated by the responses of endometrial grafts in the anterior chamber of the eye, and by oestrin excretion.

6. Lactation in males, though not usually occurring from influence of the graft alone, does occur when extracts of pituitary are administered; corpus luteum secretions are not essential for lactation.

7. The physiological capacity of the hypophysis differs in the two sexes. This difference appears to express a sex-limited character.

8. Ovarian grafts in experimental cryptorchid males reduce the intensity of testis hormone secretion by virtue of oestrin interference with hypophysis activity.

9. Sex hormone antagonism appears not to exist, at least in post-pubertal animals. The reciprocal gonad-hypophysial influences that exist in the normal organism for the control of the state of function of the gonads and accessory organs, and to some extent the anterior lobe of the hypophysis, offer a logical explanation for the activity of ovarian grafts and mammary gland responses.

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